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(54) Title: INSECT CONTROL WITH A HYPERSENSITIVE RESPONSE ELICITOR

(57) Abstract

The present invention relates to a method of controlling insects on plants. This involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to control insects on the plant or plants produced from the plant seed. Alternatively, transgenic plants or transgenic plant seeds transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to control insects.

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# INSECT CONTROL WITH A HYPERSENSITIVE RESPONSE ELICITOR

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/039,226, filed February 28, 1997.

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## FIELD OF THE INVENTION

The present invention relates to the control of insects.

10

## BACKGROUND OF THE INVENTION

The introduction of synthetic organic pesticides following World War II brought inestimable benefits to humanity and agricultural economic profitability. The widescale deployment of DDT resulted in the complete riddance, from entire countries, of serious public pests such as malaria mosquitoes. The use of DDT, other organochlorines, and, later, organophosphorus and carbamate materials was enthusiastically adopted into control programs despite occasional warnings about the hazard of unilateral approaches to pest control.

The development of new pesticides and the increasing amounts of pesticides used for pest control are closely correlated with the development of pest resistance to chemicals. The number of pesticide resistant species has greatly increased since the adoption of DDT in 1948. As a result, by the 1980s, the number of reports of pesticide resistance for arthropod pests was listed as 281, for plant pathogens 67, and for weeds 17. These numbers have steadily increased to the present day. Thus, the need for biological control agents, especially those with broadbase activity is especially important.

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The present invention is directed to overcoming these problems in the art.

#### SUMMARY OF THE INVENTION

5

The present invention relates to a method of insect control for plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or plant seeds under conditions effective to control insects on the plants or plants grown from the plant seeds.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to control insects on plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to control insects.

The present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants,

preventing insects from colonizing host plants,  
preventing colonizing insects from releasing phytotoxins,  
etc. The present invention also prevents subsequent  
disease damage to plants resulting from insect infection.

5 As a result, the present invention provides  
significant economic benefit to growers.

#### BRIEF DESCRIPTION OF THE DRAWINGS

10 Figure 1 is a plot for the field study of  
Example 4.

Figure 2 shows the mean number of pepper fruit  
lost to bacterial soft rot for control, Kocide, Kocide +  
Maneb, and hypersensitive response elicitor ("harpin")  
15 treatments predisposed by European Corn Borer.

Figure 3 shows the mean number of pepper fruit  
(all sizes) damaged by European Corn Borer for control,  
Kocide, Kocide + Maneb, and hypersensitive response  
elicitor ("harpin") treatments.

20 Figure 4 shows the mean number of large pepper  
fruit damaged by European Corn Borer for control, Kocide,  
Kocide + Maneb, and hypersensitive response elicitor  
("harpin") treatments.

#### DETAILED DESCRIPTION OF THE INVENTION

25 The present invention relates to a method of  
insect control for plants. This method involves applying  
a hypersensitive response elicitor polypeptide or protein  
30 in a non-infectious form to all or part of a plant or a  
plant seed under conditions to control insects on plants  
or plants grown from the plant seed. Alternatively, the  
hypersensitive response elicitor protein or polypeptide  
can be applied to plants such that seeds recovered from  
35 such plants are themselves effective to control insects.

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As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to control insects.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention can correspond to hypersensitive response elicitor polypeptides or proteins derived from a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora pythium*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

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The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein. In addition, seeds in accordance with the present invention can be recovered from plants which have been treated with a hypersensitive response elicitor protein or polypeptide in accordance with the present invention.

In one embodiment of the present invention, the hypersensitive response elicitor polypeptides or proteins can be isolated from their corresponding organisms and applied to plants or plant seeds. Such isolation procedures are well known, as described in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994); He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin<sub>ps</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby incorporated by reference. See also pending U.S. Patent

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Application Serial Nos. 08/200,024 and 08/062,024, which are hereby incorporated by reference. Preferably, however, the isolated hypersensitive response elicitor polypeptides or proteins of the present invention are  
5 produced recombinantly and purified as described below.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes  
10 encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seeds cells. In these embodiments, the hypersensitive response elicitor  
15 polypeptide or protein is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause  
20 the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a  
25 hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial  
30 application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or  
35 their seeds which are not susceptible to the disease



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carried by the bacteria. For example, *Erwinia amylovora* causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato plants or seeds to enhance growth without causing disease in that species.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	
	1				5					10					15		
15	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	
				20					25					30			
	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	
20				35				40					45				
	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	
		50					55					60					
25	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	
	65					70				75					80		
	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	
				85						90					95		
30	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	
				100					105					110			
	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	
35			115					120					125				
	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	
		130					135					140					
40	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	
	145					150				155					160		
	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	
				165					170					175			
45	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	
				180					185					190			
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	
50			195					200					205				
	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	
				210			215						220				

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Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
 225 230 235 240  
 5 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
 245 250 255  
 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
 260 265 270  
 10 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
 275 280 285  
 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
 290 295 300  
 15 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
 305 310 315 320  
 20 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
 325 330 335  
 Asn Ala

25 This hypersensitive response elicitor polypeptide or  
 protein has a molecular weight of 34 kDa, is heat stable,  
 has a glycine content of greater than 16%, and contains  
 substantially no cysteine. The *Erwinia chrysanthemi*  
 hypersensitive response elicitor polypeptide or protein  
 30 is encoded by a DNA molecule having a nucleotide sequence  
 corresponding to SEQ. ID. No. 2 as follows:

CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CCGTATTCTGA CACCGTTACG 60  
 35 GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC 120  
 GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG 180  
 40 CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG 240  
 TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG 300  
 CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACG GGCGGGAATG 360  
 45 ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC 420  
 CGATCATTA AATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT 480  
 CACCGTCGGC GTCACCTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG 540  
 50 GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA 600  
 AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC 660  
 55 TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCAGCG TGGATAAACT 720  
 GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT 780  
 GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC 840

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	TTTCGGCAAT GCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA	900
	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
5	CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG	1080
10	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
	GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACCTCAGC ACCCACGTAG ACGGTAACAA	1260
15	CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
	TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
	GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
20	CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
	TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
25	GGCTGTCGTC GCGGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
	ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC	1680
30	TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
	ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC	1800
	GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
35	CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTCTATCC GCCCCTTAG	1920
	CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG	1980
40	GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
	AAAATAGGGC AGTTTTTTCG TGGTATCCGT GGGGTGTTC GGCCTGACAA TCTTGAGTTG	2100
	GTTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T	2141
45		

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

50	Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser	1 5 10 15
55	Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln	20 25 30
	Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn	35 40 45
60	Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met	50 55 60

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Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175  
 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365  
 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380  
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400  
 Gly Ala Ala

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This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
20	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
25	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
30	GGCGGCAACA ATACCACTTC AACACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
35	CCGATGCAGC AGCTGCTGAA GATGTTGAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
40	CTCCTTGGCA ACGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
	GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
45	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT	900
	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG	960
	GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC	1020
50	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC	1080
	AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
55	ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200

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GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260  
 CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

5

The hypersensitive response elicitor  
 polypeptide or protein derived from *Pseudomonas syringae*  
 has an amino acid sequence corresponding to SEQ. ID.  
 No. 5 as follows:

10

	Met	Gln	Ser	Leu	Ser	Leu	Asn	Ser	Ser	Ser	Leu	Gln	Thr	Pro	Ala	Met	
	1				5					10					15		
15	Ala	Leu	Val	Leu	Val	Arg	Pro	Glu	Ala	Glu	Thr	Thr	Gly	Ser	Thr	Ser	
				20					25					30			
	Ser	Lys	Ala	Leu	Gln	Glu	Val	Val	Val	Lys	Leu	Ala	Glu	Glu	Leu	Met	
			35					40					45				
20	Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala	
		50					55					60					
	Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val	
25	65					70					75					80	
	Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe	
				85					90					95			
30	Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met	
				100					105					110			
	Thr	Gln	Val	Leu	Asn	Gly	Leu	Ala	Lys	Ser	Met	Leu	Asp	Asp	Leu	Leu	
			115					120					125				
35	Thr	Lys	Gln	Asp	Gly	Gly	Thr	Ser	Phe	Ser	Glu	Asp	Asp	Met	Pro	Met	
		130					135					140					
	Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro	
40	145					150				155						160	
	Lys	Pro	Asp	Ser	Gly	Ser	Trp	Val	Asn	Glu	Leu	Lys	Glu	Asp	Asn	Phe	
					165				170					175			
45	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile	
				180					185					190			
	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly	
			195				200						205				
50	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser	
		210					215					220					
	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	
55	225					230				235						240	
	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	
					245				250						255		

20 This hypersensitive response elicitor polypeptide or  
protein has a molecular weight of 34-35 kDa. It is rich  
in glycine (about 13.5%) and lacks cysteine and tyrosine.  
Further information about the hypersensitive response  
elicitor derived from *Pseudomonas syringae* is found in  
25 He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas  
*syringae* pv. *syringae* Harpin<sub>PSS</sub>: a Protein that is  
Secreted via the Hrp Pathway and Elicits the  
Hypersensitive Response in Plants," Cell 73:1255-1266  
(1993), which is hereby incorporated by reference. The  
30 DNA molecule encoding the hypersensitive response  
elicitor from *Pseudomonas syringae* has a nucleotide  
sequence corresponding to SEQ. ID. No. 6 as follows:

35	ATGCAGAGTC	TCAGTCTTAA	CAGCAGCTCG	CTGCAAACCC	CGGCAATGGC	CCTTGTCTCTG	60
	GTACGTCCTG	AAGCCGAGAC	GACTGGCAGT	ACGTCGAGCA	AGGCGCTTCA	GGAAGTTGTC	120
	GTGAAGCTGG	CCGAGGAAct	GATGCGCAAT	GGTCAACTCG	ACGACAGCTC	GCCATTGGGA	180
40	AAACTGTTGG	CCAAGTCGAT	GGCCGAGAT	GGCAAGGCGG	GCGGCGGTAT	TGAGGATGTC	240
	ATCGTGCGC	TGGACAAGCT	GATCCATGAA	AAGCTCGGTG	ACAAC TTCGG	CGCGTCTGCG	300
	GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
45	AAGTCGATGC	TCGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
	GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCGC	ACAGTTTCCC	480
	AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAAC TTCCT	TGATGGCGAC	540
50	GAAACGGCTG	CGTTCCGTTT	GGCACTCGAC	ATCATTGGCC	AGCAACTGGG	TAATCAGCAG	600

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	AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC	660
	AACAATCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CCGTGACAGC	720
5	GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA	780
	TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGAC CCGTACGTCG	840
10	GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG	900
	GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT	960
	GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA	1020
15	GCCTGA	1026

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

	Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln	1 5 10 15
25	Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser	20 25 30
	Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile	35 40 45
30	Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly	50 55 60
35	Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala	65 70 75 80
	Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser	85 90 95
40	Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met	100 105 110
	Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala	115 120 125
45	Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val	130 135 140
50	Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala	145 150 155 160
	Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly	165 170 175
55	Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly	180 185 190
60	Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala	195 200 205



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Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 5 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 10 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 15 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 20 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 25 Gln Ser Thr Ser Thr Gln Pro Met  
 340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 8 as follows:

30 ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60  
 AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120  
 35 GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180  
 GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240  
 AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300  
 40 GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA 360  
 GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420  
 45 GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480  
 GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540  
 GCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600  
 50 GCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660  
 GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720  
 55 CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG 780  
 ATGATGCAGC AAGGCGGCCT CGGCGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840  
 60 GGCAACGCCT CGCCGGCTTC CGGCGGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900  
 GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960  
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020  
 65 ACGCAGCCGA TGTA 1035

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Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a  
 5 Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor  
 10 polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

15	Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Val	Ala	Ile	Ile	Ala	Ile	Leu	Ala
	1				5					10					15	
	Ala	Ile	Ala	Leu	Pro	Ala	Tyr	Gln	Asp	Tyr						
				20					25							

20 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins  
 25 determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid  
 30 sequence corresponding to SEQ. ID. No. 10 as follows:

35	Ser	Ser	Gln	Gln	Ser	Pro	Ser	Ala	Gly	Ser	Glu	Gln	Gln	Leu	Asp	Gln
	1				5				10					15		
	Leu	Leu	Ala	Met												
				20												

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

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subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide is shown in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

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The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under which genes encoding an elicitor are expressed. Cell-free  
5 preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of  
10 full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional  
15 molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

20 As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave  
25 elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the  
30 primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for

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increase and expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of a useful fragment is the popA1 fragment of the hypersensitive response elicitor polypeptide or protein from *Pseudomonas solanacearum*. See Arlat, M., F. Van Gijsegem, J.C. Huet, J.C. Pemollet, and C.A. Boucher, "PopA1, a Protein Which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), which is hereby incorporated by reference. As to *Erwinia amylovora*, a suitable fragment can be, for example, either or both the polypeptide extending between and including amino acids 1 and 98 of SEQ. ID. NO. 3 and the polypeptide extending between and including amino acids 137 and 204 of SEQ. ID. No. 3.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

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The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage

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and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

5               Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transection of plasmids into cells infected with virus.

              Suitable vectors include, but are not limited  
10 to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning  
15 Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes,"  
Gene Expression Technology vol. 185 (1990), which is  
20 hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard  
25 cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

              A variety of host-vector systems may be  
30 utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;  
35 microorganisms such as yeast containing yeast vectors;

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mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these  
5 vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events  
10 control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby  
15 promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further,  
20 procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient  
25 translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-  
30 end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and



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Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of  
5 expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For  
10 instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*,  
15 *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription  
20 of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is  
25 necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

30 Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein  
35 synthesized, respectively. The DNA expression vector,

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which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9  
5 bases 5' to the initiation codon (ATG) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of  
10 coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the  
15 hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system.  
20 Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds  
25 to control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet,  
30 parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of  
35 suitable ornamental plants are: *Arabidopsis thaliana*,

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*Saintpaulia*, *petunia*, *pelargonium*, *poinsettia*,  
*chrysanthemum*, *carnation*, and *zinnia*.

The present invention is effective against a wide variety of insects. For purposes of the present invention, insects (Phylum Arthropoda, Class Insecta) also encompasses Phylum Mollusca (snails and slugs represented by the spotted garden slug, banded slug, marsh slug, and gray garden slug), Class Arachnida (mites), and Phylum Nematoda (roundworms or nematodes). The host range for some of these pests is extensive. For example, the European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plants species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larvae and adult feeding pests which feed on and damage a wide variety of vegetables and small fruits include the following:

Vegetables -- seed corn maggot, rice armyworm, alfalfa leafhopper, aster leafhopper, beet armyworm, cabbage looper, cabbage root maggot, Colorado potato beetle, corn earworm, cotton or melon aphid, diamondback moth, fall armyworm, flea beetles (various adult species feed on cabbage, mustard, and other crucifers, cucumber, eggplant, tobacco, potato, melon, and spinach), green peach aphid, onion maggot, onion thrips, pepper maggot, pickleworm (melon worm), potato leafhopper, potato stem borer, potato and corn stalk borer, striped cucumber beetle, spotted cucumber beetle, northern and western corn root worm, thrips, tarnish plant bug, tobacco aphid, tomato pinworm, tomato mole cricket, and rootknot nematode; Small fruits -- meadow spittlebug, strawberry bud weevil, strawberry root weevil, tarnish plant bug, and strawberry spider mites; Grapes -- grape berry moth, grape cane gallmaker, climbing cutworms, grape leafhoppers (three species), and grape cane girdler.

Collectively this group of insects and allied species

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represents the most economically important group of pests for vegetables, small fruit, and grape production worldwide.

The method of the present invention involving  
5 application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive  
10 response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, dusting, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application  
15 embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, dusting, or injection. Other suitable application procedures can be envisioned by those skilled in the art  
20 provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or  
25 artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor  
30 protein or polypeptide to control insects on the plants. Such propagated plants may, in turn, be useful in producing seeds or propagules (e.g., cuttings) that produce plants capable of insect control.

The hypersensitive response elicitor  
35 polypeptide or protein can be applied to plants or plant

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seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, herbicide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced according to procedures well known in the art, such as by

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biolistics or *Agrobacterium* mediated transformation. Examples of suitable hypersensitive response elicitor polypeptides or proteins and the nucleic acid sequences for their encoding DNA are disclosed *supra*. Once  
5 transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in control of insects on the plant. Alternatively,  
10 transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions  
15 effective to control insects. While not wishing to be bound by theory, such growth enhancement may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used  
20 in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including  
25 hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, dusting, and immersion. Similarly, after plants have been propagated from the transgenic  
30 plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.). The transgenic plants of the present  
35 invention are useful in producing seeds or propagules

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(e.g., cuttings) from which plants capable of insect control would be produced.

#### EXAMPLES

5

Example 1 - Controlling the Spread of Aphids From Colonized or Infested Tobacco

10 Two to three lower leaves (at position 4) of a tobacco plant were infiltrated with hypersensitive response elicitor at a concentration of 20  $\mu\text{m}/\text{ml}$ . Another tobacco plant infiltrated with 5 mM potassium phosphate buffer was used as a control. Any visible aphids on these two plants were then killed. The two  
15 plants were placed on a lab bench with a light on at night. Five days after infiltration of hypersensitive response elicitor, a heavily aphid-infected tobacco plant was moved from the greenhouse to the lab bench. The aphid-infected plant was placed close to and between the  
20 hypersensitive response elicitor-treated plant and the buffer-infiltrated plant with many of the leaves of the uninfected plants overlapping with those of the infected plant to facilitate movement of the aphids from the infected plant. The number of aphids on hypersensitive  
25 response elicitor- and buffer-treated plants were counted once everyday for about 10 days. The result is shown in Table 1.

Table 1 - Harpin Induced Tobacco Resistance To Aphid Infection

A	B		C		D		E		F		G		H		I	
	Day 1		Day 2		Day 3		Day 6		Day 7		Day 8		Day 9		Day 10	
Leaf Position	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
1	7	5	17	9	8	7	8	9	7	11	4	13	10	22	17	32
2	3	7	12	5	12	19	8	12	24	39	22	39	17	26	4	22
3	3	7	3	12	3	27	1	>50	4	>50	12	>50	2	>50	4	>50
4	4	10	3	12	2	>50	1	>50	0	>50	0	>50	0	>50	0	>50
5	2	6	1	8	0	10	0	18	0	22	0	22	0	22	0	26
6	2	0	2	4	0	4	0	11	0	22	0	20	0	18	1	26
7	0	0	0	0	0	4	0	14	0	14	2	14	0	10	0	10
8	1	12	1	4	0	8	1	24	0	22	0	22	0	32	0	32
9	0	0	0	5	0	5	0	7	0	12	0	9	0	9	0	7
10	0	0	0	3	0	3	0	13	0	15	0	15	0	12	0	10
11	0	0	0	5	0	5	0	6	0	11	0	11	0	11	0	19
12	0	2	0	3	0	11	0	11	0	6	0	6	0	8	0	8
13	0	0	0	0	0	0	0	2	0	2	0	6	0	6	0	11
14	0	0	0	0	0	0	0	1	0	6	0	8	0	21	0	14
15	0	0	0	0	0	7	0	9	0	32	0	32	0	32	0	22
16	0	0	0	0	0	0	1	0	0	0	0	2	0	4	0	11
17	0	0	0	0	0	1	0	5	0	5	0	4	0	24	4	22
18	0	0	2	0	0	0	0	1	0	1	0	1	0	18	0	16
19	1	11	1	11	0	8	4	17	4	17	0	17	2	13	0	7
20	0	0	0	0	0	0	0	0	0	0	0	0	4	6	4	14
Total	23	60	42	81	25	>169	24	>260	39	>337	38	>341	37	>388	34	>409

H: Harpin-induced plant  
C: Control plant



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From these results, it is clear that the hypersensitive response elicitor-treated plant has many fewer aphids than the buffer-treated control plant, suggesting that the aphids did not like to colonize on the hypersensitive response elicitor-treated plants. At the lower three leaves, there was a substantial number of aphids even in the hypersensitive response elicitor-treated plant. Since infiltration of hypersensitive response elicitor started from leaf 4, this indicates that the hypersensitive response elicitor-generated signal for insect-resistance can only effectively travel upward to the top of the tobacco plant.

It was also observed that aphids died 2 days after they moved to the hypersensitive response elicitor-treated plant.

**Example 2 - Colonization of Aphids in Hypersensitive Response Elicitor-Treated Tobacco Plants**

From Example 1, it was observed that there were many dead aphids on the hypersensitive response elicitor-treated tobacco leaves. To further confirm this observation, aphids were artificially inoculated on a hypersensitive response elicitor-treated tobacco plant. The number of living and dead aphids were counted once every day for 4 days.

**Hypersensitive Response Elicitor Treatment and Aphid Inoculation:** Two lower leaves of tobacco plants were infiltrated with hypersensitive response elicitor at a concentration of 20  $\mu\text{g/ml}$ . After 24 hours, tissue necrosis was observed. Seven days after hypersensitive response elicitor infiltration, aphids from an infested (or colonized) plant were transferred to the three upper leaves of the hypersensitive response elicitor-treated plant.

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Table 2 summarizes the results of this example. It shows that, after two days, most of the inoculated aphids were dead and some of them moved away from the hypersensitive response elicitor-treated plant; however, the number of the inoculated aphids in the control plant remained about the same.

Table 2 - Number of Colonized Aphids in Control and Harpin-Treated Tobacco Plants

	A	B	C	D	E	F
	Leaf	Day 0	Day 1	Day 2	Day 3	Day 4
		H C	H C	H C	H C	H C
1	1	23 22	18 20	6 20	0 19	0 21
2	2	26 27	14 27	3 25	0 25	0 28
3	3	31 25	12 26	2 22	1 24	0 20
Total	Total	80 74	44 73	11 67	1 68	0 69

The numbers in the table are live aphids  
 H: Harpin-induced plant  
 C: Control plant

Example 3 - Tobacco Seedlings Generated from Harpin-Soaked Seeds are Resistant to Aphid Infection

About 80 tobacco seeds (*Nicotiana tabacum* L. 'Xanthi') were soaked in harpin solution (about 25 µg/ml of 5 mM potassium phosphate buffer, pH 6.5) for about 16 hours. Then, the harpin-soaked seeds were sowed in a 6" pot with artificial soil. The same treatment using a 5 mM potassium phosphate buffer without harpin was used as a control. The pots were incubated in a growth chamber at a temperature of 25°C with 14 hour day light. Twenty days after sowing, the size of the tobacco seedlings treated with harpin was significantly greater

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than that of control plants. Twenty seedlings subjected to each treatment were transplanted to 8" pots 28 days after sowing. The seedlings were then incubated in a growth room at a temperature of about 23°C using 14 hour day lights. By the time the seedlings were transplanted, aphid infection was observed in the control tobacco seedlings, but not in the harpin-treated seedlings. The source of aphid infection was previously infected adult tobacco plants in the same growth chamber. In the growth room, 7 precolonized adult tobacco plants were placed around the seedlings being tested to serve as a natural source of aphids. Seven days after the seedlings were transplanted, the number of aphids in each tobacco seedlings was counted. As shown in Table 3, 17 out of 20 control plants were infected by aphids with the number of aphids varying between 1 to 13. However, only 2 out of 20 harpin-treated plants were infected by the aphids. This indicates that tobacco plants from harpin-treated seeds are far more resistant to the aphid infection than control plants.

Table 3 - Tobacco Plants Generated From Harpin-Soaked Seeds Are Resistant To Aphid Infection

5	<u>Control</u>		<u>Harpin-Treated</u>	
	<u>Plant No.</u>	<u>Number of Aphids</u>	<u>Plant No.</u>	<u>Number of Aphids</u>
	1	4	1	0
	2	2	2	10
	3	11	3	0
	4	11	4	0
10	5	4	5	0
	6	13	6	0
	7	3	7	0
	8	5	8	0
	9	11	9	0
15	10	1	10	0
	11	3	11	0
	12	4	12	0
	13	4	13	0
	14	0	14	0
20	15	12	15	0
	16	2	16	0
	17	0	17	0
	18	2	18	0
	19	0	19	0
25	20	2	20	0
	Total	94		10

Example 4 - Field Study Regarding The Effect Of  
Hypersensitive Response Elicitor  
Application On Insect Control

5 An experiment was conducted at the Homer C. Thompson Vegetable Research Farm located in Freeville, NY. The experimental design was a randomized complete block with four replications, with 8 plants per rep, using single rows on plastic, with 22 inch spacing

10 between plants. A single inoculated spreader row of peppers ran the length of the plot between the two treatment rows to provide inoculum for the target disease of bacterial leaf spot of pepper (*Xanthomonas campestris* pv. *vesicatoria*, pepper race). See Figure 1. Upwind

15 and across the road from the pepper trial was a commercial field of dent corn which provided a natural source of European corn borer during the season. The pepper variety "Jupiter" was selected because of its strong susceptibility to bacterial leaf spot. Pepper

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seedlings were transplanted to the field on day 0. Bacterial inoculum was introduced into the plot by two means. Previously infected "Jupiter" seedlings were transplanted to the spreader row on day 26 and the spreader row was additionally inoculated on day 38 with *Xanthomonas campestris* pv. *vesicatoria* pepper race in order to provide more disease pressure for the peppers rows on either side.

The first application of hypersensitive response elicitor or harpin was made on day 23, before any inoculum was introduced or spread had occurred. A total of four treatments were tested: (1) water sprayed control; (2) Kocide at 3 lb/A; (3) Kocide at 1 lb + Manex fungicide at 1.2 qt/A; and (4) Harpin. The copper fungicide Kocide and the Kocide + Manex (maneb) fungicide are standard materials recommended for bacterial leaf spot control in pepper. Kocide is manufactured by Griffin Corp., Valdosta, GA, while Manex is produced by Crystal Chemical Inter-America, Houston, TX. All treatments were applied with a CO<sub>2</sub> pressurized boom sprayer at approximately 40 psi with 21.5 gal/A being delivered through four TeeJet XR 11003 flat fan nozzles spaced 20 inches apart. This provided excellent foliar coverage. Following initial harpin treatment, all treatments were applied weekly until the experiment was concluded. No additional pesticides, including insecticides, were applied. The first appearance of disease in the test plants was on day 54. Two pepper harvests were made on day 61 and day 97. Data taken included the incidence (i.e. number of plants infected with bacterial leaf spot) per treatment, total number and weight of fruit harvested by category (large, medium, small, and unmarketable), and the total number of fruit showing European corn borer damage expressed as frass or unharvestable because of fruit breakdown by bacterial

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soft rot *Erwinia carotovora* subsp. *carotovora*. The involvement of European corn borer became evident at about day 50. Consequently, the amount of soft rot for all treatments was recorded at the day 57 and day 97 harvests. Similarly, it became apparent on the day 57 harvest that European corn borer damage could also be assessed by larval feeding (i.e. frass) on pepper fruit. The European corn borer overwinters as the last larval instar, and, in the spring, the larvae pupate. Adults from the multi-generation strain emerge in late May to early June and again in August. If a single generation strain is present, then the emergence will peak in July. However, in some fields of the Northeast, single and multi-generation strains may be present together. Female moths fly into susceptible crops to lay their eggs, and each female may lay up to 500 eggs during its lifespan. After hatching, the tiny borers crawl to protected areas on the plant to feed, which in the case of pepper, is under the calyx attachment of the pod to the stem. They later borer into the pod, allowing bacteria to enter and rapidly multiply in the moist and humid environment within the pod. Bacterial soft rot can destroy the pod in a manner of days. Differences in European corn borer damage and infestations among treatments was recorded at the time of the second harvest. Data were analyzed and significance established by one-way analysis of variance.

Bacterial leaf spot foliar infections occurred throughout the plots, but the amount of disease did not allow for any significant differences. Final disease ratings were made on day 97. The harpin treatment provided control equivalent to the commercial treatments of Kocide or Kocide + Manex, and all were better than the water-sprayed control. The number of European corn borer (ECB) damaged fruit that were rotting on the plants on day 97 were recorded; they could not be harvested because

of watery collapse. The harpin treated plots had fewer rotting pepper pods, and although not significantly different from the other treatments ( $P=0.229$ ), the amount of protection provided with the harpin sprays was evident (See Figure 2). Another indication of the amount of damage caused by European corn borer feeding was the number of fruit showing feeding damage or frass. The harpin treated fruit had substantially less fruit damage across all fruit sizes ( $P=0.076$ ), when compared with all other treatments (See Figure 3). The number of large fruit with borer damage was significantly reduced ( $P=0.048$ ) when sprayed with harpin (See Figure 4).

The benefit of using harpin to reduce the damage caused by the European corn borer was reflected in two ways. First, substantially less bacterial soft rot leading to loss of fruit in the field was noted when harpin was applied weekly. Secondly, the number of fruit with direct borer feeding (i.e. frass) was much lower in harpin treated plots than all other treatments. The greatest impact of harpin treatment on economic factors was the greater production of undamaged fruit across all size categories, and the greater yield of healthy large fruit which have the highest dollar value.

25    Example 5 -    Control of Aphid from Foliar Application  
                                 of HP-1000™ Hypersensitive Response  
                                 Elicitor to Cotton.

Cotton aphids (*Aphis gossypii*) leave a "honeydew" deposit that contaminates the lint and reduces crop value. A field trial to determine the effect of HP-1000™ Hypersensitive Response Elicitor from *Erwinia amylovora* (Eden Bioscience Corp., Bothell, Wash.) on cotton (var. Acala) was seeded in replicated (4X) plots (3.2 x 25 feet) in a randomized complete block design. Treatments were HP-1000™ at 20, 60, and 80 µg/ml (a.i.) and a chemical insecticide, Asana XL® (DuPont Agricultural

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Products, Wilmington, DE), at 8 oz./ac. Foliar treatments were applied beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a back-pack sprayer. Aphid counts were made immediately  
5 prior to spray applications at 14, 28, 35, and 42 days after the first treatment (DAT 1). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates, and ten leaves per plot at the final sampling date.

10 At 14 DAT 1 (i.e. on day 14), aphid counts were relatively low across all treatments, but by 28 DAT 1 (two sprays applied) (i.e. on day 28) the number of aphids per leaf were significantly greater in Asana XL<sup>®</sup> treated plots compared to the HP-1000<sup>™</sup> treated plots  
15 (Table 4). By 35 DAT 1 (three sprays applied) (i.e. on day 35), aphid counts had risen for all treatment rates, yet aphid counts per leaf was still significantly lower for HP-1000<sup>™</sup> treated cotton compared to the Asana XL<sup>®</sup> treatment. Finally, at 42 DAT 1 (four sprays applied)  
20 (i.e. on day 42), the number of aphids per leaf had increased to a level that threatened to overwhelm all treatments, including the chemical standard insecticide. At this point, Pravado<sup>®</sup> aphicide (Bayer Corporation, Agricultural Division, Kansas City, MO) was applied to  
25 all plots to eradicate aphids from all treatments and the trial was continued for crop yield only.

These data indicate that cotton treated with HP-1000<sup>™</sup> deterred light to moderate aphid pressure and that this effect was significantly better than a standard  
30 chemical insecticide, Asana XL<sup>®</sup>.



Table 4 - Aphid Count per Leaf on Cotton After Treatment with Asana XL<sup>®</sup> or HP-1000<sup>™</sup>

Treatment	Rate <sup>2</sup>	Number of Aphids Per Leaf <sup>1</sup> No. Sprays Applied/Days After Treatment			
		1/14DAT1	2/28DAT1	3/35DAT1	4/42DAT1
Asana XL <sup>®</sup>	8 oz/ac	0.2 a	32.2 a	110.0 a	546.9 a
HP-1000 <sup>™</sup>	20 µg/ml	0.2 a	7.8 b	22.9 b	322.1 a
HP-1000 <sup>™</sup>	60 µg/ml	0.1 a	4.9 b	34.6 b	168.3 a
HP-1000 <sup>™</sup>	80 µg/ml	0.0 a	2.7 b	25.8 b	510.2 a

<sup>1</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05. <sup>2</sup>Rate for Asana XL<sup>®</sup> is for formulated product, rate for HP-1000<sup>™</sup> is for active ingredient (a.i.).

Example 6 - Control of Strawberry Spider Mites by Foliar Application of HP-1000<sup>™</sup> to Cotton.

Mites cause foliar damage to cotton thus reducing potential crop yield. To assess potential mite control of HP-1000<sup>™</sup>, cotton (var. Acala) was seeded in replicated (4X) field plots (3.2 x 25 feet) in a randomized complete block field trial. Treatments included HP-1000<sup>™</sup> at 20, 60, and 80 µg/ml and a chemical insecticide for mites, Zephyr<sup>®</sup> (Novartis, Greensboro, NC), at 6 oz./ac. HP-1000<sup>™</sup> treatments were applied at 14 day intervals using a back-pack sprayer beginning when the crop was at three true leaves. Zephyr<sup>®</sup> was applied once, on the same date as the first application of HP-1000<sup>™</sup>. A pretreatment evaluation for strawberry spider mites (*Tetranychus turkestanii*) was made immediately before the first spray and again at 4, 7, 14, and 28 days after the first treatment (DAT 1).

Mite populations were determined by collecting twenty-five randomly chosen cotton leaves per plot. All leaves were brushed with a mite brushing machine and dislodged mites were uniformly distributed onto a

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rotating glass plate, pretreated with a wetting agent to which they adhered. The number of motile adult mites were counted under a 30X binocular microscope. This figure was then converted to a per leaf unit.

5 A count of living or motile adult mites per leaf at the five assessment times did not appear to show significant treatment effects at any of the evaluation times (Table 5).

10 Table 5 - Number of Adult Motile Mites per Leaf After Treatment with Zephyr<sup>®</sup> or HP-1000<sup>™</sup>.

Treatment	Rate <sup>2</sup>	Number of motile mites per leaf				
		evaluation timing				
		0DAT1	4DAT1	7DAT1	14DAT1	28DAT1
Zephyr <sup>®</sup>	6 oz/ac	3.4	0.2	0.4	0.0	0.0
HP-1000 <sup>™</sup>	20 µg/ml	2.0	0.6	0.3	0.0	0.0
HP-1000 <sup>™</sup>	60 µg/ml	3.7	0.5	0.1	0.2	0.1
HP-1000 <sup>™</sup>	80 µg/ml	3.0	1.4	0.4	0.0	0.0

<sup>2</sup>Rate for Zephyr<sup>®</sup> is for formulated product, rate for HP-1000<sup>™</sup> is for active ingredient (a.i.).

25 However, using the method of Henderson et al., "Tests With Acaracides Against Brown Wheat Mites," J. Econ. Ent. Vol. 48(2):157-61 (1955), which is hereby incorporated by reference ("Henderson"), to calculate percent mortality

30 revealed the mite control was different between treatments. (Table 6).

Henderson's Method is defined as:

$$\text{Percent Mortality} = \frac{\text{Ta} \times \text{Cb}}{1 - \text{Tb} \times \text{Ca}} \times 100$$

5      where;      Ta = Number of motile mites counted after treatment,

10                Tb = Number of motile mites counted prior to treatment,

                 Ca = Number of mites in the control (check) after treatment of the test plots, and

15                Cb = Number of mites in the control (check) plot before treatment of the test plots.

20

When percent mortality was calculated at 4 DAT 1, mite control from treatment with HP-1000™ was over two times greater compared to Zephyr® (Table 6). By 7 DAT 1, mite control was still substantially better from HP-1000™ treatment than for Zephyr®. At 14 DAT 1, mite control for HP-1000 at 80 µg/ml reached its maximum at just under 84%, roughly comparable to that seen for the Zephyr® treatment. For the remaining 14 days, mite control by HP-1000™ treatments tended to decline relative to the Zephyr®. Treatment with Zephyr® reached 100% mite control by 28 DAT 1 (Table 6).

25

30

Table 6 - Control of Motile Adult Mites on Cotton from Treatment with HP-1000™ as Measured by Henderson's Method.

Treatment	Rate <sup>2</sup>	Percent control of motile mites <sup>1</sup>			
		evaluation timing			
		4DAT1	7DAT1	14DAT1	28DAT1
HP-1000™	20 µg/ml	56.6	76.5	68.4	66.7
HP-1000™	60 µg/ml	57.1	50.0	78.5	40.0
HP-1000™	80 µg/ml	53.6	77.9	83.8	60.0
Zephyr®	6 oz/ac	28.0	66.7	89.9	100.0

<sup>1</sup>Percent control calculated using Henderson's method (1955).

<sup>2</sup>Rate for Zephyr® is for formulated product, rate for HP-1000™ is for active ingredient (a.i.).

These data indicate that the mode of action for mite control is different between HP-1000™ and Zephyr®. Complete control by treatment with Zephyr® was not achieved until 28 DAT. Weekly treatments with HP-1000™ resulted in relatively "steady" mite control throughout the 28 day evaluation period. This suggests HP-1000™ may trigger an internal insect resistance process fundamentally different than chemical insecticide activity.

**Example 7 - Reduced Feeding Activity of Mole Cricket in Tomato from Foliar Application of HP-1000™**

Fresh market tomatoes (var. Agri-set) were planted at 12-inch spacing in 25 foot rows replicated 5 times in a randomized completed block design field trial. This disease control trial was not specifically designed to assess insect resistance from treatment with HP-1000™. Foliar applications of HP-1000™ at 20 and 40 µg/ml were applied beginning at first true leaves and repeated at 7 day intervals for 8 sprays. Additional treatment included a standard commercial fungicide mixture (Bravo®

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(Zeneca Ag Products, Wilmington, DE)+Manex™+Kocide®) for control against bacterial blight disease. After the first four spays were applied, a field evaluation was made to determine and the number of plants damaged (girdled) by feeding of mole cricket (*Scapteriscus vicinus*, scudder). Data presented in Table 7 indicates that HP-1000™ treated plants had considerably less girdling from mole cricket feeding. Continued evaluations of this trial were not possible due to complete crop loss from virus infection.

Table 7 - Reduced Stem Girdling of Tomatoes by Mole Cricket from Application of HP-1000™.

Treatment	Rate <sup>1</sup>	No. Plant girdled <sup>2</sup>	% chg. Vs. UTC
UTC	----	15	----
Bravo®	1 quart/ac	12	-20
+Manex™	2 lbs/ac		
+Kocide®	1.5 pints/ac		
HP-1000™	40 µg/ml	4	-73
HP-1000™	40 µg/ml	7	-53

<sup>1</sup>Rates for Bravo®, Manex® and Kocide® are for formulated product; rates for HP-1000 are for a.i.

<sup>2</sup>Average number of plants from 50 plants per replicate.

**Example 8 - Reduced Feeding Activity of Army Worm in Rice from Foliar Application of HP-1000™**

Rice seed (var. M-202) was presoaked for 24 hours in a solution of HP-1000™ at a concentration of 20 µg/ml (a.i.). Treated rice was then seeded into randomized (5X) field plots 10 x 15 feet. An untreated control treatment was also included; no foliar sprays were applied to this trial. Observation at 41 days after planting revealed significant damage to leaves due to feeding of armyworm (*Spodoptera praefica*) larvae. To

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quantify the damage, one hundred randomly selected tillers were taken from HP-1000™ treated as well as untreated plots. Samples were ranked for damage according to the following scale:

5

- 1 = no tiller leaves damaged  
2 = one tiller leaves with feeding damage  
3 = two tiller leaves with feeding damage  
4 = three tiller leaves with feeding damage  
5 = four or all tiller leaves with feeding damage

10

15

Results from these rankings were then analyzed for treatment differences. Data presented in Table 8 indicate that rice plants treated with HP-1000™ had significantly less feeding damage than the UTC plants.

HP-1000™ treated rice was virtually untouched by armyworm feeding.

Table 8 - Reduced Armyworm Feeding on Rice After Seed Soak Treatment with HP-1000™.

25

Treatment	Rate <sup>1</sup>	Median Rating <sup>2</sup>
UTC		3 (two tiller leaves damaged)
HP-1000™	20 µg/ml	1 (no tiller leaves damaged)

30

<sup>1</sup>Rate is for active ingredient applied (a.i.). <sup>2</sup>Difference in median values among the two groups is statistically different according to Mann-Whitney Rank Sum Test, P = 0.0001.

35

Example 9 - Reduced Feeding Activity of Aphids in Tobacco from Foliar Application of HP-1000™

40

Tobacco seedlings were treated with two foliar sprays of HP-1000™ at rates of 15, 30, and 60 µg/ml (a.i.). The first application was made to seedlings, the second approximately 42 days later after transplanting

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into replicated (3X) field plots. Two days after the second application, counts for tobacco worm and aphid were made. Data presented in Table 9 illustrate that HP-1000™ treatment substantially reduced the amount of feeding activity from both tobacco worm and aphid.

Table 9 - Reduced Feeding Activity of Tobacco Worm and Aphid from Treatment with HP-1000™ on Tobacco.

Treatment	Rate	No. tobacco worms/100 plants	Percent of plants with aphids feeding
UTC		20	13
HP-1000™	15 µg/ml	10	7
HP-1000™	30 µg/ml	4	4
HP-1000™	60 µg/ml	10	7

Example 10 - Tomato Seedlings Treated with HP-1000™ Show Tolerance to Nematodes.

Tomato seedlings (var. Rutgers) were germinated in flats and grown for four weeks before transplanting into pots, two plants per pot, replicated eight times. At transplanting, seedlings were treated with HP-1000™ at 25 µg/ml via root soaking. One week after transplanting, each pot was inoculated with approximately 10,000 root knot nematode, RKN, (*Meloidogyne hapla*) eggs. Thereafter, weekly root drenches of HP-1000™ continued until four weeks. After four weeks, one plant in each pot was evaluated for root weight and the number of galls (i.e. infections sites on the roots from nematode parasitism). The remaining plants were then treated with four weekly foliar sprays of HP-1000™ (25 µg/ml a.i.). After all treatments had been applied, these plants were then evaluated for root weight, shoot weight, and number of fruit per plant. Four weeks after inoculation, the

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number of galls per plant was slightly higher for HP-1000™ treated plants than for the control plants, yet the shoot weight was significantly greater for HP-1000™ treated plants (Table 10).

5

**Table 10 - Number of Galls and Shoot Weight of RKN-inoculated Tomatoes After Treatment with HP-1000™.**

10

Treatment	Rate <sup>1</sup>	No. Galls/plant	Shoot wt. <sup>2</sup> (g/plant)
UTC	----	427	32.8 a
HP-1000™	25 µg/ml	507	39.5 b

15 <sup>1</sup>Rate is for amount of active ingredient, a.i. <sup>2</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 This indicated that even though nematodes were infecting HP-1000™ treated plants, plant growth was still enhanced by the HP-1000™ treatment. Eight weeks after inoculation, (four additional foliar HP-1000™ sprays applied) shoot weight was still significantly higher for  
 25 HP-1000™ treated plants vs. control plants also inoculated with RKN and the average number of fruit per plant was numerically higher in the HP-1000™ treated plants (Table 11).

30 **Table 11 - Average Shoot Weight and Average Number of Fruit per Plant of RKN-inoculated Tomatoes After Treatment with HP-1000™.**

35

Treatment	Rate <sup>1</sup>	Shoot wt. <sup>2</sup> (g/plant)	No. Fruit/plant
UTC	----	69.9 a	0.875
HP-1000™	25 µg/ml	89.8 b	1.25

40 <sup>1</sup>Rate is for amount of active ingredient, a.i. <sup>2</sup>Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.



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These results indicate that treatment with HP-1000™ appears to enable the tomato plants to "tolerate" the negative impact of the nematodes.

5    **Example 11** -    **Effect of *Erwinia amylovora* Hypersensitive Response Elicitor on Repellency of Cucumbers to Striped Cucumber Beetles.**

10                    The hypersensitive response elicitor protein  
was encoded by the *hrpN* gene of *Erwinia amylovora* ("harpin")  
was produced by fermentation of the cloned gene in a  
high-expression vector in *Escherichia coli*.  
High-pressure liquid chromatography analysis of the  
cell-free elicitor preparation was used to determine its  
15    harpin content. Treatment dilutions were prepared in  
water. Harpin was applied as a foliar spray to caged,  
cucumber plants, Marketmore 76, lot #1089, to assess its  
ability to repel the striped cucumber beetle, *Acalymma*  
*vittatum* (Fabricius). Harpin from *E. amylovora* was  
20    applied in water at 0, 5, and 10 mg/l to cucumber plants  
21-days after sowing seed in the greenhouse (plants had  
both cotyledons and 6-8 fully expanded leaves/plant).  
Each concentration was applied to three plants per block,  
and the treatments were replicated three times. Seven  
25    days after treatment, a mean of 4.6 adult beetles per  
plant were introduced manually. The insects were allowed  
to feed for 7 days before feeding damage to the plants  
was evaluated. The number of cotyledons and the number  
of leaves showing any damage from beetle feeding was  
30    determined. A rating scale of 0-6 (where 0 = no obvious  
feeding; 1 = < 15% damage; 2 = < 25% damage; 3 = < 50%  
damage; 4 = > 50% damage; 5 = > 75% damage; and 6 = leaf  
desiccated or dead due to feeding) was used to estimate  
the extent of damage from beetle feeding on the  
35    cotyledons and leaves.

Table 12 summarizes the effect of  
hypersensitive response elicitor protein concentration on

insect damage. The mean percent of damaged cotyledons was in direct proportion to the harpin concentration, whereas the damage to leaves was inversely proportional to harpin concentration.

5

Table 12 - Effect of Treating Cucumber Foliage with a Hypersensitive Response Elicitor on the Subsequent Feeding Damage Caused by the Striped Cucumber Beetle (*Acalymma vittatum* [Fabricius]).

10

Harpin Concentration (mg/l)	Cotyledons <sup>1</sup> Percent Damaged	Leaves	
		Percent Damaged	Damage Rating
0	34	42	5.42
5	50	18	3.40
10	67	5	3.20

15

20

<sup>1</sup>Nine plants per treatment in three blocks of three each. Damage was assessed on a 0-6 scale where 0 = no feeding injury, and 6 = cotyledons and leaves dead because of extensive beetle feeding.

25

More damage probably occurred on the lower cotyledons, because most of the foliar harpin spray was directed to the upper foliage and it was assumed that more harpin activity would be found in the upper leaves (upward or systemic harpin effect). The cotyledons were thus very attractive for beetle feeding. Less damage occurred on leaves of plants that had been treated with the higher concentration of harpin. Thus, the effectiveness of the treatment on leaves increased as the harpin concentration increased.

35

The effect of harpin is significant for two reasons: 1) damage from beetle feeding on cucurbits, especially cucumbers, melons, pumpkins, and summer and winter squash, is reduced, because treatment of cucumber

40

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with harpin resulted in the plants becoming less attractive (repulsive or repellent) to insect feeding and 2) damage from the bacterial wilt disease is likely to be reduced because these same beetles vector the bacterium responsible for the disease. By preventing feeding, transmission of the bacterium responsible for the disease could be reduced or eliminated. This study shows that harpin may be used to decrease insect damage caused by beetle feeding. Thus, the number of applications of insecticides to particularly insect-sensitive cucurbits might be reduced or eliminated with harpin.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: INSECT CONTROL WITH A  
HYPERSENSITIVE RESPONSE ELICITOR
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
  - (B) STREET: P.O. Box 1051, Clinton Square
  - (C) CITY: Rochester
  - (D) STATE: New York
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 60/039,226
  - (B) FILING DATE: 28-FEB-1997
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Goldman, Michael L.
  - (B) REGISTRATION NUMBER: 30,727
  - (C) REFERENCE/DOCKET NUMBER: 19603/1522
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (716) 263-1304
  - (B) TELEFAX: (716) 263-1600

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 338 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
 1 5 10 15  
 Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
 20 25 30  
 Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr  
 35 40 45  
 Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu  
 50 55 60  
 Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser  
 65 70 75 80  
 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
 85 90 95  
 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp  
 100 105 110  
 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln  
 115 120 125  
 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met  
 130 135 140  
 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly  
 145 150 155 160  
 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly  
 165 170 175  
 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu  
 180 185 190  
 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala  
 195 200 205  
 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val  
 210 215 220  
 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
 225 230 235 240  
 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
 245 250 255  
 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
 260 265 270  
 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
 275 280 285  
 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
 290 295 300

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Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
 305 310 315 320  
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
 325 330 335  
 Asn Ala

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2141 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCTGA CACCGTTACG	60
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTGCGCGCA ATCCGGCGTC	120
GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG	180
CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCAGCAGAG	240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTGTTGAACT GGCAGGAATG	360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CCGACGCGCC	420
CGATCATTA GATAAAGGCG GCTTTTTTTT TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
CACCGTCGGC GTCACCTCAGT AACAAATATC CATCATGATG CCTACATCGG GATCGGCGTG	540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC	660
TCAGGGACTG AAAGGACTGA ATTCGCGCGC TTCATCGCTG GGTTCAGCG TGGATAAACT	720
GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT	780
GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
TTTCGGCAAT GCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA	900
TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG	1080

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CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
GGCTGTCGTC GCGGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC	1680
TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC	1800
GTGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG	1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG	1980
GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG	2100
GTTCGTCATC ATCTTCTCC ATCTGGGCGA CCTGATCGGT T	2141

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 403 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser
1				5					10					15	
Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln
			20					25					30		

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Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45  
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60  
 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175  
 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365



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Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400

Gly Ala Ala

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA	420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
CTCCTTGGCA ACGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT	900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG	960
GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGCAA GCCGCAGTAC	1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC	1080
AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCACAA AGCCAAGGGC	1140

- 56 -

ATGATCAAAA GGCCCATGGC GGGTGATAACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200  
 GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260  
 CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 341 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160  
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175  
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190  
 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205

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Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220  
 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240  
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255  
 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270  
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335  
 Asn Gln Ala Ala Ala  
 340

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1026 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTTG	60
GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC	120
GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180
AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC	240
ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG	300
GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC	360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC	420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC	480
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC	540

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GAAACGGCTG CGTTCCGTTT GGCACCTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG	600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC	660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC	720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA	780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCGCGCAGAC CGGTACGTCG	840
GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG	900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT	960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA	1020
GCCTGA	1026

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 344 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln	1	5	10	15
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser	20	25	30	
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile	35	40	45	
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly	50	55	60	
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala	65	70	75	80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser	85	90	95	
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met	100	105	110	
Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala	115	120	125	
Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val	130	135	140	

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Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160  
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175  
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190  
 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205  
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 Gln Ser Thr Ser Thr Gln Pro Met  
 340

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1035 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC	60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180

- 60 -

GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC	240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG	420
GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC	480
GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC	540
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT	600
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC	660
GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC	720
CAGGGCGGCC TCACCGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG	780
ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC	840
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT	900
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC	960
GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG	1020
ACGCAGCCGA TGTA	1035

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Val	Ala	Ile	Ile	Ala	Ile	Leu	Ala
1				5					10					15	
Ala	Ile	Ala	Leu	Pro	Ala	Tyr	Gln	Asp	Tyr						
			20					25							

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser	Ser	Gln	Gln	Ser	Pro	Ser	Ala	Gly	Ser	Glu	Gln	Gln	Leu	Asp	Gln
1				5					10					15	

Leu	Leu	Ala	Met
			20

## WHAT IS CLAIMED:

1. A method of insect control for plants comprising:
  - 5 applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to control insects on the plant or plants grown from the plant seed.
- 10 2. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*,
  - 15 *Xanthomonas*, *Phytophthora*, and mixtures thereof.
3. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.
  - 20
4. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.
- 25 5. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.
- 30 6. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.



7. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

5 8. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to a *Phytophthora* species.

10 9. A method according to claim 1, wherein the plant is selected from the group consisting of dicots and monocots.

15 10. A method according to claim 9, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash,  
20 pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

25 11. A method according to claim 9, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

30 12. A method according to claim 1, wherein plants are treated during said applying which is carried out by spraying, injection, or leaf abrasion at a time proximate to when said applying takes place.

35 13. A method according to claim 1, wherein plant seeds are treated during said applying which is

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carried out by spraying, injection, coating, dusting, or immersion.

14. A method according to claim 1, wherein the  
5 hypersensitive response elicitor polypeptide or protein  
is applied to plants or plant seeds as a composition  
further comprising a carrier.

15. A method according to claim 14, wherein  
10 the carrier is selected from the group consisting of  
water, aqueous solutions, slurries, and powders.

16. A method according to claim 14, wherein  
the composition contains greater than 0.5 nM of the  
15 hypersensitive response elicitor polypeptide or protein.

17. A method according to claim 14, wherein  
the composition further contains additives selected from  
the group consisting of fertilizer, insecticide,  
20 fungicide, nematocide, and mixtures thereof.

18. A method according to claim 1, wherein the  
hypersensitive response elicitor polypeptide or protein  
is in isolated form.  
25

19. A method according to claim 1, wherein the  
hypersensitive response elicitor polypeptide or protein  
is applied as bacteria which do not cause disease and are  
transformed with a gene encoding the hypersensitive  
30 response elicitor polypeptide or protein.

20. A method according to claim 1, wherein the  
hypersensitive response elicitor polypeptide or protein  
is applied as bacteria which cause disease in some plant  
35 species, but not in those subjected to said applying, and

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contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

21. A method according to claim 1, wherein  
5 said applying causes infiltration of the polypeptide or protein into the plant.

22. A method according to claim 1, wherein  
said applying is effective to prevent insects from  
10 contacting plants to which the hypersensitive response elicitor is applied.

23. A method according to claim 22, wherein  
plants are treated during said applying.  
15

24. A method according to claim 22, wherein  
plant seeds are treated during said applying, said method  
further comprising:

planting the seeds treated with the  
20 hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted  
in the soil.

25. A method according to claim 1, wherein  
said applying is effective to cause insects to depart  
from plants to which the hypersensitive response elicitor  
is applied.

26. A method according to claim 25, wherein  
30 plants are treated during said applying.

27. A method according to claim 25, wherein  
plant seeds are treated during said applying, said method  
35 further comprising:

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planting the seeds treated with the  
hypersensitive response elicitor in natural or artificial  
soil and

propagating plants from the seeds planted  
5 in the soil.

28. A method according to claim 1, wherein  
said applying is effective to kill insects proximate  
plants to which the hypersensitive response elicitor is  
10 applied.

29. A method according to claim 28, wherein  
plants are treated during said applying.

15 30. A method according to claim 28, wherein  
plant seeds are treated during said applying, said method  
further comprising:

planting the seeds treated with the  
hypersensitive response elicitor in natural or artificial  
20 soil and

propagating plants from the seeds planted  
in the soil.

31. A method according to claim 1, wherein  
25 said applying is effective to interfere with insect  
larval feeding on plants to which the hypersensitive  
response elicitor is applied.

32. A method of insect control for plants  
30 comprising:

providing a transgenic plant or plant seed  
transformed with a DNA molecule encoding a hypersensitive  
response elicitor polypeptide or protein and

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growing the transgenic plants or  
transgenic plants produced from the transgenic plant  
seeds under conditions effective to control insects.

5           33. A method according to claim 32, wherein  
the hypersensitive response elicitor polypeptide or  
protein corresponds to that derived from a pathogen  
selected from the group consisting of *Erwinia*,  
10 *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures  
thereof.

34. A method according to claim 33, wherein  
the hypersensitive response elicitor polypeptide or  
protein corresponds to that derived from *Erwinia*  
15 *chrysanthemi*.

35. A method according to claim 33, wherein  
the hypersensitive response elicitor polypeptide or  
protein corresponds to that derived from *Erwinia*  
20 *amylovora*.

36. A method according to claim 33, wherein  
the hypersensitive response elicitor polypeptide or  
protein corresponds to that derived from *Pseudomonas*  
25 *syringae*.

37. A method according to claim 33, wherein  
the hypersensitive response elicitor polypeptide or  
protein corresponds to that derived from *Pseudomonas*  
30 *solanacearum*.

38. A method according to claim 33, wherein  
the hypersensitive response elicitor polypeptide or  
protein corresponds to that derived from *Xanthomonas*  
35 *campestris*.

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39. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a *Phytophthora* species.

5

40. A method according to claim 32, wherein the plant is selected from the group consisting of dicots and monocots.

10

41. A method according to claim 40, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

42. A method according to claim 40, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

43. A method according to claim 32, wherein a transgenic plant is provided.

44. A method according to claim 32, wherein a transgenic plant seed is provided.

30

45. A method according to claim 32, further comprising:

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applying the hypersensitive response  
elicitor polypeptide or protein to the propagated plants  
to effect insect control.

5                   46. A method according to claim 32, wherein  
said insect control prevents insects from contacting  
plants.

10                   47. A method according to claim 32, wherein  
said insect control causes insects to depart from  
transgenic plants.

15                   48. A method according to claim 32, wherein  
said insect control kills insects.

49. A method according to claim 32, wherein  
said insect control interferes with insect larval feeding  
on plants.





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MELON/PEPPER/SQUASH PLOT 1996  
NIA AT FREEVILLE FARM  
OVERALL FIELD DIMENSIONS 215' X 96'

MELONS

1. HMS 2608
2. PI157082
3. PI511890
4. PI140471
5. SUPERSTAR
6. TAM UVALDE
7. STARSHIP
8. SWEETIE #6
9. EARLY SUGAR SHAW
10. MIAMI
11. GOLDEN BEAUTY
12. SAVOR
13. SATICOY
14. EARLIGOLD
15. PASSPORT
16. DAIMIEL
17. ACAPULLO
18. BANANA
19. CHILTON PVP
20. CHILTON SELECT
21. GULF COAST PVP
22. AURORA

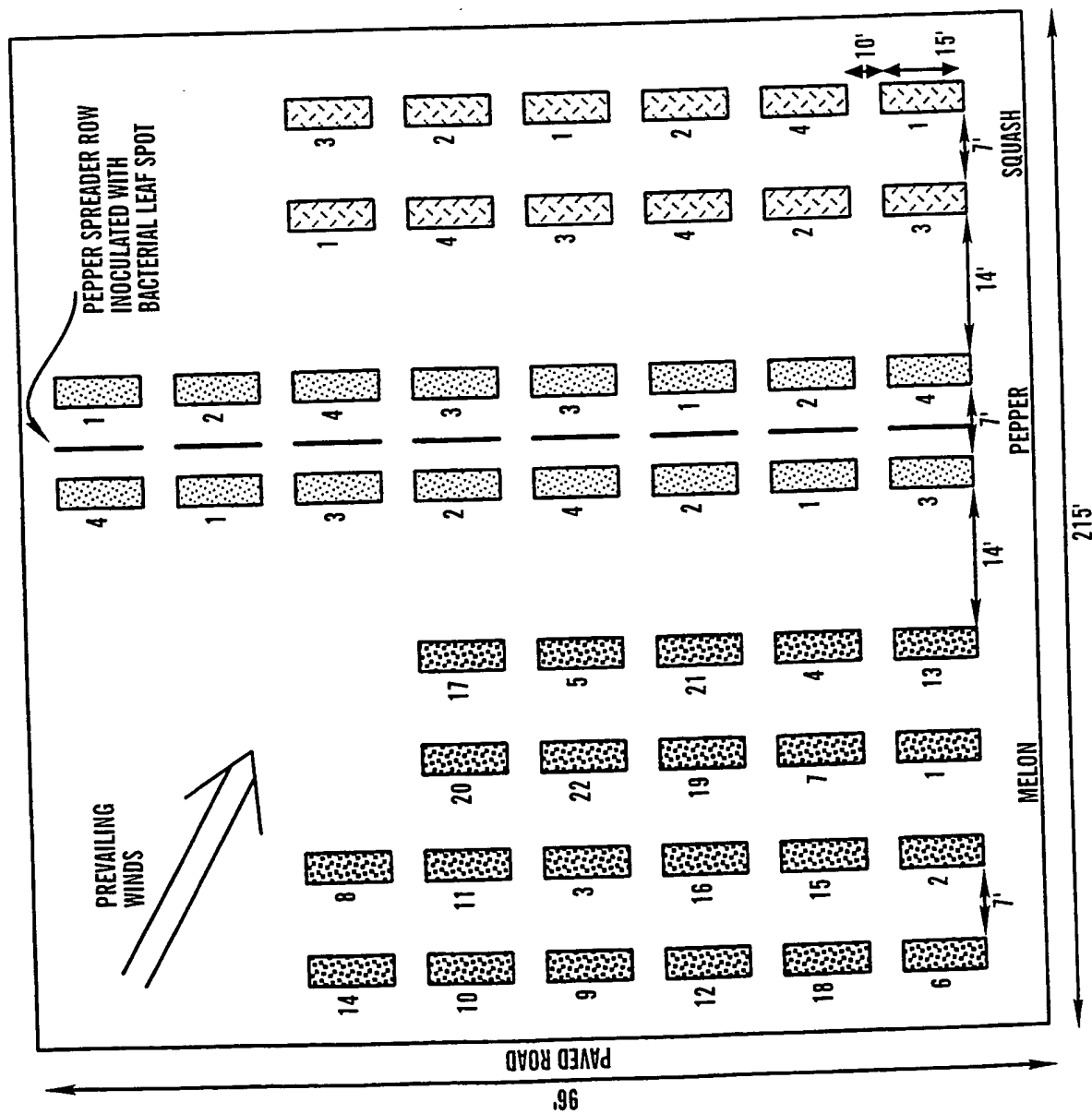
PEPPERS

1. CONTROL
2. KOCIDE, 3lb/A
3. KOCIDE, 1lb/A + Manex, 1.2 qt/A
4. HARPIN

SQUASH

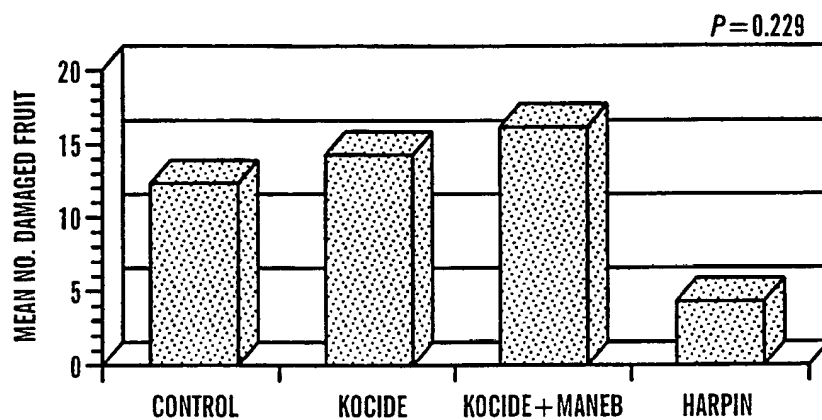
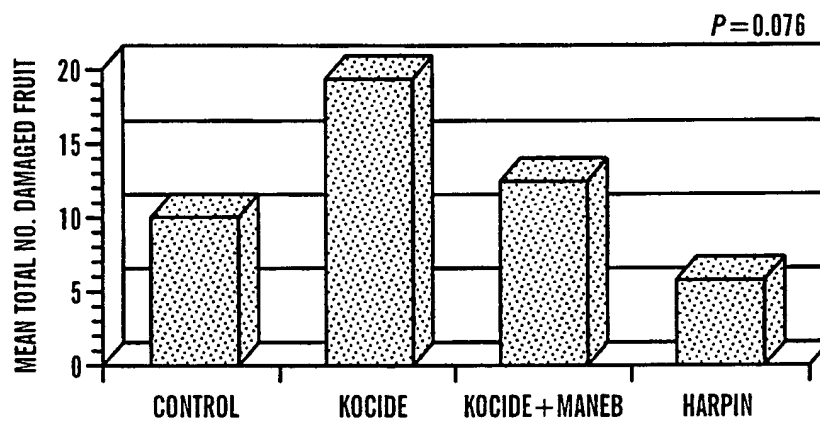
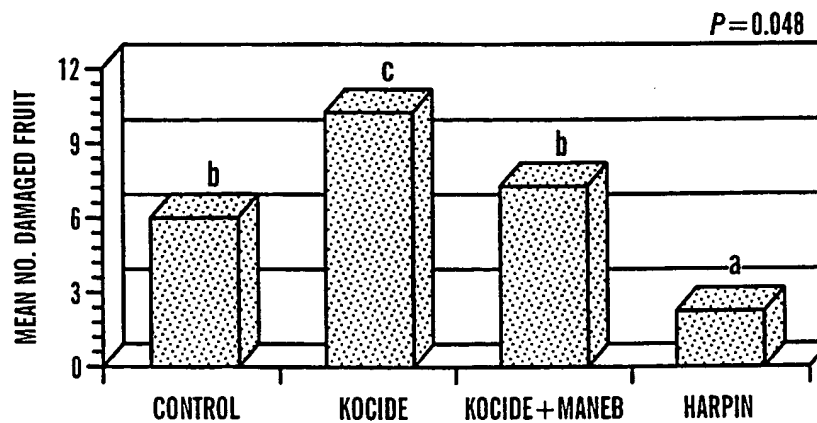
1. WALTHAM BUTTERNUT
2. NICKLOW'S DELIGHT
3. C. MARTINESEI
4. B GENE VARIETY

FIG. 1





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**FIG. 2****FIG. 3****FIG. 4**



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/03604

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01G 1/00; A01H 1/00, 1/04, 5/10; C12N 5/04, 5/10

US CL : 435/118; 800/200, 205, 250, 255

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/118; 800/200, 205, 250, 255

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	INBAR et al. Elicitors of plant defensive systems reduce insect densities and disease incidence. Journal of Chemical Ecology, January 1998, Vol. 24, No. 1, pages 135-149, see entire document.	1-49

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*g* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 MAY 1998

Date of mailing of the international search report

23 JUN 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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Telephone No. (703) 308-4916

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/03604

### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG (AGRICOLA, CRIS, BIOSIS, SCISEARCH, MEDLINE), STN (CAPLUS)

Search Terms: HR, hypersensitive response, insect resist?, alkaloid?, phytoalexin?, salicylic acid, PR protein?, transgenic plant?, pathogen-induced resistance, inventor's names